

# CLAIMS

1. A human  $\beta$ -globin mutant polypeptide comprising the amino acid sequence of the normal human  $\beta$ -globin modified by (i) the substitution or deletion of Cys at positions 93 and 112, and (ii) the substitution of a Cys  
5 for a non-Cys amino acid at one other position in the polypeptide.
2. A mutant polypeptide according to claim 1 wherein Cys is substituted for one of the amino acids at the following positions:
  - (a) Ser at position 9;
  - (b) Asn at position 80; or
  - 10 (c) Lys at position 17.
3. A mutant polypeptide according to claim 1 wherein Cys is substituted for Ser at position 9.
4. A mutant polypeptide according to claim 3 having the amino acid sequence SEQ ID NO:4.
- 15 5. A nucleic acid sequence encoding a mutant polypeptide according to claim 4.
6. A nucleic acid according to claim 4 which comprises the nucleotide sequence SEQ ID NO:2.
7. A vector comprising a promoter operably linked to a nucleic  
20 acid sequence according to claim 6, capable of directing the expression of a mutant human  $\beta$ -globin polypeptide.
8. A host cell transformed with a vector according to claim 7.

- 28 -

9. A method for producing a human  $\beta$ -globin mutant polypeptide comprising:

growing a culture of transformed host cells of claim 8 under conditions conducive to the expression of said polypeptide by said  
5 host cells.

10. A modified human hemoglobin comprising a  $\beta$ -globin polypeptide according to claim 1.

11. A modified human hemoglobin comprising a  $\beta$ -globin polypeptide according to claim 2.

10 12. A modified human hemoglobin comprising a  $\beta$ -globin polypeptide according to claim 3.

13. A modified human hemoglobin comprising a  $\beta$ -globin polypeptide according to claim 4.

14. A modified human hemoglobin according to any of claims 10-  
15 13 comprising a mutant human  $\alpha$ -globin polypeptide comprising the amino acid sequence of normal human  $\alpha$ -globin modified by the substitution or deletion of Cys at position 104.

15. A modified human hemoglobin according to claim 13 comprising a mutant human  $\alpha$ -globin polypeptide comprising the amino  
20 acid sequence of normal human  $\alpha$ -globin modified by the substitution or deletion of Cys at position 104, and further modified by either the substitution of Cys for Ala at position 71, or the substitution of Cys for Ala at position 53.

- 29 -

16. A modified human hemoglobin comprising:

a mutant human  $\alpha$ -globin polypeptide comprising the amino acid sequence of normal human  $\alpha$ -globin modified by the substitution of Cys at position 104 by a non-Cys amino acid;

5 a mutant human  $\beta$ -globin polypeptide comprising the amino acid sequence of normal human  $\beta$ -globin modified by the substitution of Cys at positions 93 and 112 by non-Cys amino acids; and

said modified hemoglobin further characterized by the substitution of Cys for the native sequence amino acid at one of the following positions:

10  $\beta$ -globin position 9;  
 $\beta$ -globin position 17;  
 $\beta$ -globin position 80;  
 $\alpha$ -globin position 71; or  
 $\alpha$ -globin position 53.

15 17. A polymeric hemoglobin comprising a modified human hemoglobin according to claim 10, 11, 12, 13 or 16, wherein adjacent hemoglobins are covalently bonded to each other by one or more disulfide bridges formed by cysteine amino acid residues.

20 18. A polymeric hemoglobin according to claim 17 wherein the modified hemoglobin is characterized by the substitution of Cys for the native sequence amino acid at  $\beta$ -globin position 9, said polymeric hemoglobin comprising seven modified hemoglobins.

19. A blood substitute comprising a polymeric hemoglobin according to claim 17.

25 20. A blood substitute comprising a polymeric hemoglobin according to claim 18.

21. A method of supplementing the oxygen-carrying capacity of a patient's blood comprising administering to the patient an effective amount of the blood substitute according to claim 19.

5 22. A method of supplementing the oxygen-carrying capacity of a patient's blood comprising administering to the patient an effective amount of the blood substitute according to claim 20.

23. A mutant human  $\alpha$ -globin polypeptide comprising the amino acid sequence of normal human  $\alpha$ -globin modified by the substitution or deletion of Cys at position 104.

10 24. A mutant human  $\alpha$ -globin polypeptide comprising the amino acid sequence of normal human  $\alpha$ -globin modified by the substitution or deletion of Cys at position 104, and further modified by either the substitution of Cys for Ala at position 71, or the substitution of Cys for Ala at position 53.